Etiology

Shasta Daisy Vascular Wilt Incited by *Acremonium strictum*

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ABSTRACT


A new vascular wilt of the Shasta daisy (Chrysanthemum maximum) in California was investigated. Symptoms included vascular browning, wilting, stunting, and unilateral chlorosis and necrosis of the lower leaves. *Acremonium strictum* was isolated consistently from the stems, roots, and occasionally petioles of diseased plants. Although rarely seen, hyphae and conidia were found in yellowed xylem vessels of diseased plants. Disease symptoms were reproduced in the greenhouse and the field, although these were not as severe as in naturally infected field stock. Symptom expression in the field was cyclic and correlated with the recovery of *A. strictum* from affected plants. The host had to be stressed by excessive soil moisture or by the onset of flowering to obtain symptoms comparable to those of field plants. The host range of *A. strictum* included monocotyledonous and dicotyledonous agricultural plants as well as several species of weeds.

Vascular wilts of Shasta daisy have been recognized for many years. Both *Fusarium* and *Verticillium* spp. have been isolated from infected plants and were accepted as causal agents of the disease although no research has been published to substantiate the relationship. Recently, another organism, *Acremonium strictum* W. Gams (5), was implicated in a new vascular wilt disease of *Chrysanthemum maximum* (Ram.) by Chase (3).

Species of the genus *Acremonium* have not been widely recognized as plant pathogens, probably because they formerly were classified in the genus *Cephalosporium*. In 1977, a new key to the group of Cephalosporium-like hyphomycetes was published (5) which facilitated accurate identification, and as a result *Acremonium* spp. have been implicated increasingly in plant diseases. Krüger (8) isolated several species of *Fusarium* as well as *A. strictum* from maize with stalk and root rot symptoms. No pathogenicity tests were performed. *A. strictum* was least frequently isolated, and its role in the disease presumably was secondary. Kang and Singh (7) identified the same fungus as a causal agent of seed rot and seedling blight of maize in India. Hesseltine and Bothast (6) also found *A. strictum* associated with an ear mold disease of maize. In addition, *A. strictum* has been isolated from ryegrass (9) and iron ore tailings (13). Nigh (10) demonstrated the parasitism of *A. strictum* on eggs of the sugar beet cyst nematode. *A. strictum* also degrades aircraft fuel (11) and is antagonistic to the plant pathogen *Leptosphaeria coniothyrium* (Fckl.) Sacc. (12). It has an extremely wide range of activities.

This research was initiated to study the epidemiology of Shasta daisy (*C. maximum 'Killian') wilt, to identify and demonstrate the pathogenicity of the causal agent, and to partially determine the host range of that organism.

MATERIALS AND METHODS

Cultural practices. Shasta daisies are grown in southern California in coastal areas where temperatures rarely exceed 33°C in the summer or drop below 0°C in the winter. The season begins in June and July when the previous year's crop is disked under, plowed, subsoiled, and disked again. The fields are sprinkled every 7–10 days to speed germination of weed seeds and decomposition of the crop debris. Soil is fumigated with a 2:1 mixture of methyl bromide and chloropicrin, at the rate of 340 kg per hectare, and covered with a polyethylene tarp (0.025 mm thick) for 7 days. After the tarp is removed, the soil is disked again, and the beds are formed by tractor with an inorganic slow-release fertilizer added in a strip to the center of each bed. In June, stock plants growing in open fields are cut back to 10 cm from the ground to facilitate new growth. Healthy appearing plants are dug up, split into divisions consisting of a vegetative shoot and its immediate roots, and stored bare rooted at 0°C for 1–3 days. This procedure greatly increases the survival of the transplants. They are then planted approximately three per meter in two rows in the beds (1.2-m centers) and sprinkled irrigated. By the end of September, all of the fields are replanted. Beginning in December, plants are lighted each night from 2200 hours to 0200 hours. The intensity of the light at the level of the foliage is adjusted to 35 lux. The plants grow vegetatively in a rosette until the additional light stimulates flowering and the stems elongate to 1 m. In January, the flowers from the first plantings are harvested. Under normal light conditions, the flowers would not be produced until late May. Flowering continues through June, but at this time most of the plants do not produce salable flowers and the fields are plowed under.

Epidemiological studies. The development of the disease was followed for 3 yr, 1976–1979. Overall symptoms were observed twice monthly. The percentage of the stems infected with *A.
strictum was determined. The degree of vascular browning at the base of a stem was rated in severity from 0 (no browning) to 4 (maximum browning). Stems from each of 50 plants were rated and an average value was calculated. The age of the tissue was standardized by selecting stems on which flowers were beginning to open. In the fall when the plants were vegetative, a shoot was substituted for a flower stem. An attempt was made to choose the same age of tissue each sampling period by selecting stems of similar size. The percentage of infection with A. strictum was determined by dividing each of 20 or 30 stems into seven pieces and taking three 2-3 mm cross-sectional pieces from each of the six cuts. The pieces were surface-disinfested for 3 min in 1.2% sodium hypochlorite, rinsed in sterile deionized water (SDW), and plated on 1.5% agar supplemented with 60 µg/ml streptomycin sulfate (WAS) medium. The percentage of pieces positive for A. strictum was recorded after incubation at 24 C for 6 days.

The distribution of the disease in California was determined by obtaining samples of plants from each of the major areas of the state where the Shasta daisy is grown commercially. The symptoms were noted and organisms were isolated from the stems by using the techniques described above.

Isolation of the causal agent. Tissue was selected from field-grown Shasta daisy plants with symptoms including wilting, vascular browning, chlorosis, and necrosis of the lower leaves. Stem, petiole, and root pieces were washed in tap water, surface-disinfested in 0.6% or 1.2% sodium hypochlorite for 0 to 10 min at 1-min intervals, rinse in SDW, tap water, or not rinsed, and placed in plates containing WAS, potato-dextrose agar (PDA), PDA containing 60 µg/ml of streptomycin sulfate (PDA-S) medium, or Tsao and Ocana’s (14) selective medium for pythiacious fungi. Plates were incubated in polyethylene bags for 4, 5, 6, 7, or 8 days at 18, 24, or 27 C. At the end of each of these incubation times the isolates were transferred to the tissues were identified and isolated. These observations were made during each month of a season to ensure the recovery of most bacteria and fungi colonizing the tissue.

Histological studies. Permanent paraffin mounts were made from stem tissue of naturally infected Killian daisies. Ten stems were washed in tap water, and cut into seven pieces from the tip of the crown in the same manner as used in the epidemiological study. Three cross-sectional pieces, 2-3 mm thick, were cut from the ends of the pieces. They were surface-disinfested and placed on WAS medium as described in the epidemiology section. From stems that yielded A. strictum on WAS medium, a piece of the remaining stem tissue (1-2 cm long) was fixed, sectioned, and stained with safranin and fast green. Healthy tissue was obtained from seedlings grown in the greenhouse and sectioned and stained as above.

Pathogenicity experiments. Pathogenicity of A. strictum and Alternaria sp. was tested by infesting UC soil mix (2) with mycelial slurries of one or both of the fungi and using noninfested soil. A single-spore isolate was grown on fresh PDA medium in petri plates under continuous light for 2 wk at 24 C. Each plate was blended with 50 ml of SDW for 15-30 sec at the high setting in a Waring Blender, and the mixture was added to 7.5-cm-diameter clay pots at the rate of one piece per 350 cc of soil. Five to 10 pots were infested for each treatment. Each pot was planted with either 10 seeds of okra (Hibiscus esculentus L., 'Clemson Spineless Pod') or 25 seeds of Killiam daisy. After 12 days in a greenhouse, the percentage germination was recorded. The height and weight of the okra plants and the presence of fungi were determined after 12 days. This experiment was repeated once with okra seed and A. strictum only and three times with Killiam daisy seed and A. strictum only.

The pathogenicity of A. strictum to seedlings in field soil was tested by collecting soil from commercial fields and allowing it to air-dry for 2 days. The soil was mixed and half of it was autoclaved for 1 hr at 121 C on each of 2 consecutive days. The soil was then mixed with an equal volume of steam-sterilized UC soil mix, and half of each of the following (autoclaved and non-autoclaved) was mixed with a mycelial slurry of A. strictum at the rate of one petri plate culture per 350 ml of soil. The remaining portion of soil was mixed with PDA medium alone at the same rate. Three-week old plants were planted singly into seven 7.5-cm-diameter clay pots for each soil treatment, and placed in groups in the greenhouse for 4 mo. In one experiment, three seedlings per each treatment were removed after 3 wk and examined. The recovery of the fungus from the plants and the development of symptoms was noted at that time. This test was performed three times.

The effect of flowering on the development of symptoms was tested as follows: 40 seedlings in 10-cm-diameter peat pots were inoculated with 5 X 10^6 conidia of A. strictum suspended in SDW and 30 additional seedlings were uninoculated. Conidial suspensions were made from a 2-wk-old culture of a single-spore isolate grown in continuous light. The conidia were counted with a hemocytometer and diluted with SDW. After 4 wk, seedlings were placed in the greenhouse for 3 mo, when half were exposed to additional light (35 lux, from 2200 hours to 0200 hours each night) and the remaining plants were maintained under normal light. After 3 mo, all of the plants were examined for vascular browning and chlorosis and isolations for A. strictum were made.

The effect of watering was tested by using seedlings of Killian daisy grown in 7.5-cm-diameter clay pots for 3 mo. A single-spore isolate of A. strictum was grown on PDA in intermittent light for 2 wk. A mycelial disk, 5 mm in diameter, was placed in a 1-cm-long cut made in the crown portion of 10 seedlings. Ten seedlings were inoculated with a disk of PDA alone. Half of the pots that received each treatment were placed in a greenhouse and watered normally while the remainder were placed in saucers and kept saturated during the test. After 4 mo, the severity of symptoms and the recovery of A. strictum were determined. Seedlings were obtained from Killian daisy seeds collected the previous season and grown in a greenhouse in UC soil mix until they were 10-12 wk old. Fifty of these were transferred to the field. Symptom development was monitored frequently and at the end of the season (June 1978) one stem from each plant was dissected and plated on WAS medium and the degree of vascular browning in that stem was noted. This test was repeated in the 1978–1979 season, with the exception that 150 seedlings were planted in an area at least 16 m from any infected field plants.

Host range of A. strictum. The host range of A. strictum was tested using oat (Avena sativa L.): sugarbeet (Beta vulgaris L. 'USH-10'); aster (Callistephus chinensis L.); Nees. 'Burpkeana Extra Early'; pyrethrum (Chrysanthemum cinerariifolium [Trev.] Vis. 'Giant'); Shasta daisy (C. maximum Ram. 'Killian' and 'Majorc', chrysanthemum (C. morifolium [Ramat.] HemsIl. 'Rainbow'); carrot (Daucus carota L. var. sativa D.C. 'Nantes Half-long'); soybean (Glycine max L. Merrill 'Harosoy'); cotton (Gossypium hirsutum L. 'SJ-2'); okra (Hibiscus esculentus L. 'Clemson Spineless Pod'); tomato (Lycopersicon esculentum L. 'Rutgers'); alfalfa (Medicago sativa L. 'Africa' and 'Moapa 69'); tobacco (Nicotiana tabacum L.); Petunia sp.; radish (Raphanus sativus L. 'Cherry Bell'); rye (Secale cereale L.); cineraria (Senecio cruentus [Mass.] D.C. 'Festival'); sorghum (Sorghum bicolor [L.] Moench. 'Wheat'); wheat (Triticum aestivum L. 'Era 1302'); and corn (Zea mays L. 'Bantam'). Seeds were surface disinfested for 45 min in a mixture composed of 0.6% sodium hypochlorite, 10% ethanol, and one drop of Triton X-100 per 100 ml of solution, rinsed in SDW, and dried for 60 min at 65 C. Seeds were planted in steam-sterilized UC soil mix in 7.5-cm-diameter clay pots and thinned to five plants per pot. Three pots of each species were inoculated 1 or 2 wk after germination by drenching the soil with a spore suspension of A. strictum at the rate of 2.5 X 10^6 conidia per plant, and one pot was treated with an equal volume of sterile water. Plants were grown in a greenhouse for 2 mo before disease symptoms were determined. Two root pieces and three stem pieces, each 1 cm long, were dissected from each plant and plated in the standard manner. This test was performed three times.

RESULTS

Epidemiological studies. The average intensity of the browning at any given time during the year was relatively consistent from one season to the next. Disease development was cyclic (Fig. 1). In the fall, vascular browning was moderate. As the days became cooler
and shorter the plants grew rapidly, and the browning was reduced and remained at a low level throughout the winter. When the plants began to flower in January and February, the browning increased. By March, all disease symptoms were expressed: stunting; unilateral chlorosis and necrosis of the lower leaves; and wilting. Disease severity increased until June when it reached a peak and most of the plants were plowed under. At that time the browning of the new growth was progressively lower than previous readings.

The percentage of infection with *A. strictum* also was cyclic, similar to the vascular browning (Fig. 1), and relatively consistent from one year to the next. Vascular browning and the presence of the fungus in the stems of the plants were positively correlated during most of the season.

The winter of 1978 was unusual; rainfall was much higher than in previous years; disease development was more rapid than usual; the first symptom development was seen only in fields of flowering plants; and later in the season, as the rainfall continued, symptoms also developed in vegetative plants.

In a survey of Shasta daisy fields in California *A. strictum* was found in all fields sampled, San Luis Rey, Vista, Colma, and Half Moon Bay. The fields were observed in March and were not artificially lighted, thus wilt symptoms usually connected with flowering were not present. However, the fungus was isolated from 100% of the plants sampled.

**Isolation of the causal agent.** Isolations from stems, roots, and petioles of diseased Shasta daisies consistently yielded high percentages (50–100%) of *A. strictum*. Other organisms occasionally isolated included *Alternaria* spp. (40–60%), *Rhizoctonia* sp. (10%), *Chaetothyrium* sp. (less than 5%), and several unidentified fungi and bacteria. The percent recovery of *Alternaria* was higher (mean = 45%) than that of the others. At no time was *Fusarium* or *Verticillium* spp. isolated. *Rhizoctonia* sp. was only recovered from the crown tissue during the summer months and was confined to the cortex. When the cortex was stripped away, the recovery of *Rhizoctonia* sp. and many other fungi decreased sharply. *Alternaria* spp. were recovered during the entire season. Disinfection of at least 1.5 hr in the mixture containing 0.6% sodium hypochlorite was necessary to remove *Alternaria* spp. from seed surfaces. Since this treatment killed all other fungi, it was not employed as a standard procedure. Although the *Alternaria* spp. isolates appeared to be contaminants or secondary invaders, their pathogenicity was tested; plants inoculated with *Alternaria* spp. alone never developed disease symptoms.

**Histological studies.** Hyphae were seen more frequently than conidia, although both were scarce. Approximately 30 plants were examined, but fungal structures were seen in only five plants. The only form of reproduction seen was blastogenous (budding of the conidia). In all of the diseased tissue, the xylem vessels were yellow or brown and appeared to contain a viscous substance. Hyphae were not seen in any other tissues of diseased plants nor in pathogen-free plants.

**Pathogenicity experiments.** *A. strictum* caused pre-emergence damping-off in both Shasta daisy and okra. Damping-off of okra was variable from one experiment to the next, ranging 20–60%, although the damping-off in Shasta daisy was similar (25%) in each

![Fig. 1](image1.png)

Fig. 1. The relationship between vascular browning and percentage of infection of field-grown Shasta daisies infected with *Acremonium strictum* for three seasons, 1976–1979.

![Fig. 2](image2.png)

Fig. 2. A. Shasta daisy seedling (*Chrysanthemum maximum*) inoculated (right) with *Acremonium strictum* and noninoculated (left), showing symptoms of vascular wilt (stunting). B. Unilateral chlorosis and necrosis of lower leaves of a noninoculated seedling.
experiment. Since okra grew faster than Shasta daisy, the stunting in terms of plant fresh weight and height was more easily detected. Reduction in the weight of inoculated plants was approximately 36% in each experiment and reduction in height was 42%. Shasta daisy seedlings growing in soil infested with A. strictum had brown lesions at the crown, reduced root systems, and enlarged brown root tips. Inoculated okra seedlings also had lesions at the crown. A strictum was reisolated only from plants grown in soil infested with that fungus. Seeds of both plants germinated in soil infested with Alternaria sp. at the same rate as seeds grown in noninfested soil. There were no symptoms of disease in the resulting plants and Alternaria sp. was not reisolated from the inoculated plants.

There was little difference in the symptom development in plants grown in nonsterile field soil in the greenhouse with or without the addition of A. strictum and autoclaved field soil to which the fungus had been added. Three weeks after inoculation, seedlings from both artificially or naturally infested soil had brown lesions at the crown and swollen, rotten root tips. Seedlings grown in autoclaved noninfested soil did not have these symptoms. The mean heights of the treatments were 6.5, 7.0, and 7.0 cm, respectively, for plants grown in soil either naturally or artificially infested, or both. The mean height of the plants grown in sterile soil was significantly greater (11.4 cm). Vascular browning in noninoculated plants was rated at 0 while the rating (range = 0-1) in plants that received the other three treatments averaged 0.4. The inoculated plants also developed unilateral chlorosis and necrosis of the lower leaves, symptoms that were not found on the noninoculated plants. A strictum was reisolated from all of the plants grown in infested soil and from nonsterile field soil, but not from plants grown in sterile field soil.

Soaking of soil with excess water increased the severity of vascular wilt symptoms in Shasta daisy. Noninoculated plants remained healthy with or without excess water. Inoculated plants with normal watering were the same size as those that were inoculated and kept wet, while treatments plants were stunted in comparison to noninoculated controls (Fig. 2). The lower leaves of inoculated plants exposed to excess water showed chlorotic and necrotic streaks and the mean vascular browning intensity in stems was 1.5 (high = 3.0). Plants watered normally did not show these symptoms. Three of 10 inoculated plants that were kept wet died, while all plants that received the other three treatments survived. A strictum was reisolated from inoculated plants, but not from noninoculated plants.

Disease symptoms were more severe in inoculated plants subjected to supplementary light, than in those grown in normal light. Young seedlings developed swollen, rotten root tips, and stunted root systems prior to being transplanted to soil, whereas noninoculated seedlings were free of these symptoms. Symptoms typical of field plants, (vascular browning, chlorosis of the leaves, and stunting) developed in the inoculated plants after 6 mo and were more severe when the plants were kept at the longer day length. Shasta daisy seedlings inoculated with A. strictum developed severe symptoms shortly after the onset of flowering. Noninoculated plants remained symptomless and A. strictum was not recovered from them. Inoculated plants in normal light had an average vascular browning rating of 0.5, while those under the increased day length had an average rating of 1.5 (range = 0.5-2.0). In addition, leaf symptoms were less severe on those plants grown under normal light than on those grown under increased day length. A strictum was reisolated from all of the inoculated plants.

In other greenhouse and lathhouse experiments, symptoms did not develop until after the plants had flowered. Brown streaks in the vascular system, unilateral chlorosis and necrosis of the lower leaves, and stunting were observed but these symptoms were indistinct and difficult to rate. Noninoculated plants did not develop any of these symptoms and A. strictum was not isolated from them, although it was isolated from inoculated plants.

In the first field plot, symptoms developed by June. They included vascular browning (mean = 1.3, range = 0-3.0), chlorosis and necrosis of the lower leaves, and stunting comparable in severity to symptoms seen in naturally infected field plants. A. strictum was reisolated from all of these plants. The results of the second field plot were not as conclusive. Experimental plants separated from field plants became infected only after 5 mo in the field. Vascular browning ranged from 0 to 1 (mean = 0.5), and slight chlorosis of the lower leaves was noted. A. strictum was reisolated from all of the plants.

Host range of A. strictum. In tests of host range, all of the plants inoculated with the fungus became infected and the pathogen was easily recovered. Symptoms (stunting and brown lesions at the crowns of the plants) developed only on okra and cotton (Malvaceae). In each of the tests the frequency of recovery of the fungus was lowest from the monocots although it was too variable to compare one species with another. Representatives from several genera of weed plants (Sonchus sp., Capsella sp., Chenopodium spp., and Physalis sp.) were inoculated with A. strictum and although no symptoms developed, the pathogen consistently was reisolated. Isolations from weeds collected from the field also yielded A. strictum in differing frequencies.

DISCUSSION

A close relationship between disease and positive isolation of A. strictum from field-grown Shasta daisy plants was demonstrated during three successive seasons. This evidence implicates A. strictum in the wilt of Shasta daisy. There appears to be a dynamic balance between the fungus and the plant which can be shifted in favor of either component at any time by several factors. Environmental and physiological factors that moderately stress the host shifted the balance in favor of the fungus and symptoms developed. When environmental conditions were within the range of a normal year, the controlling factor in disease development was the physiological stress of flower production and harvesting. Symptoms did not usually appear when the plants were vegetative. Plants were monitored during a 2-mo period and plants of the same age had the same degree of symptom development. The influence of flowering on symptom expression has been noted in several diseases caused by similar organisms (1,4), supporting our conclusion that the stage of plant development was important in the disease development in Shasta daisy wilt.

Disease development changed on two occasions, in response to a freeze and also excessive rainfall. In these instances symptoms developed on plants approximately 3 mo earlier than normal. When the stress was removed, plants grew more vigorously and symptom development slowed. In most cases, the stage of plant development influenced symptoms even under stress conditions and they were most severe in flowering plants. However, after 2 mo of excessive rainfall, vegetative plants began to show marked disease symptoms. Thus, environmental stress appeared to override natural resistance in the vegetative state. Late-season high temperature stress appeared to favor the disease.

As a general rule the higher the stress on the plant the more severe the disease will become. This rule fails only at the close of the season when both the browning in the stems and the recovery of the fungus from them decreases. An inverse relationship between plant stress and the disease exists at this time, which reveals an important characteristic of this host-parasite interaction. The fungus is not a vigorous pathogen and requires a certain level of stress on the host before it can develop extensively. However, if the host is overstressed, the fungus can no longer develop at the maximum rate and it also declines. A very close association has evolved between the host and the parasite.

The presence of hyphae and conidia of A. strictum within the vessels of diseased Shasta daisies provided further evidence for the involvement of this fungus in the wilt disease. A. strictum induced all of the symptoms of Shasta daisy wilt in both greenhouse and field tests, although the severity of these symptoms, particularly vascular browning and leaf chlorosis, was not always comparable to those in field plants. It also incited symptoms in seedlings (swollen root tips, root stunting, and root rotting) in both laboratory and greenhouse experiments which were not typical in the field. The ability of this fungus to incite both the typical field symptoms in adult plants and seedling symptoms in
the same plant establishes the connection of these symptoms. If seeds were used to propagate this crop, it is probable that the symptoms described here on seedlings also would be found in the field. This was verified by the production of typical seedling symptoms on plants from seeds planted in naturally infested field soil, and by isolations that yielded only A. strictum. The variability in the expression of typical field symptoms following inoculation with A. strictum can be attributed to two factors: genetic variation of seedlings, and the relationship of the fungus to the host. Pathogen-free Killian daisies propagated from seeds were used for experimental purposes. Each seedling responded to infection a little differently and in most seedlings symptoms were not as severe as in field stock. The second factor was the necessity for stressing the infected host before symptoms developed. The less stressful conditions for plant growth in a greenhouse or lathhouse favor plant growth over that of the fungus most of the time. Thus, symptom expression was slight on greenhouse-grown plants compared to that in field plants which are more severely stressed. That the plants must be stressed before maximum symptom expression can occur was verified experimentally in the greenhouse by showing that excessive soil moisture and induction of flowering increase the intensity of vascular discoloration and chlorosis of the lower leaves.

Most plant pathogens are restricted in the number of plants which they can effectively parasitize and usually do not infect animals as well. A. strictum was capable of infecting both monocots and dicots, although symptoms rarely developed as a result of that infection. The likelihood that weeds act as a reservoir for the fungus is quite high and must be considered in designing a control program. The ability of this plant pathogen to infect and parasitize nematode eggs also must be recognized. Nigh (10) isolated the same fungus from the eggs of Heterodera schachtii A. Schm. (sugar beet cyst nematode) and demonstrated its role as an active oviparous. Isolates of A. strictum from nematode eggs were identical to those from daisies and both proved to be pathogenic on both hosts.

LITERATURE CITED